IECN 2024 Conference

The 4th International Electronic Conference on Nutrients

16–18 October 2024 | Online

MDPI

The Role of Melatonin in Enhancing Intermittent Fasting-Induced Nutritional Ketosis for Treating Metabolic Dysfunction in Obese Mice

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Materials and Methods

Experimental Design

For the study, 40 healthy adult male Swiss albino mice of similar weight and age were used and divided in four groups:

Group 1: CONTROL - NON-OBESE (10 animals), received a normal diet during the study, were not induced with nutritional ketosis, and were not treated with melatonin.

Group 2: OBESE (10 animals), were fed a diet enriched with margarine, casein, and bread for 3 weeks to nutritionally induce obesity; they were not induced with nutritional ketosis and were not treated with melatonin.

Group 3: OBESE KETOSIS UNTREATED (10 animals), were fed a diet enriched with margarine, casein, and bread for 3 weeks to nutritionally induce obesity; they were induced with nutritional ketosis through intermittent fasting for an additional 3 weeks but were not treated with melatonin.

Group 4: OBESE KETOSIS TREATED, were fed a diet enriched with margarine, casein, and bread for 3 weeks to nutritionally induce obesity; they were induced with nutritional ketosis through intermittent fasting for another 3 weeks and were treated with melatonin, administered daily via oral gavage with a melatonin solution. The melatonin solution was administered at a dose of 50 mg/kg body weight/day and was prepared by dissolving the substance in a minimal amount of absolute ethanol, then diluting it with distilled water.

The mice were kept in special cages with continuous access to food and water, except during the period of nutritional ketosis induction for Groups 3 and 4, at a controlled temperature of +20°C, without any disturbances to the light-dark cycle.

At the end of the three weeks allocated to inducing obesity, the body mass of all animals was measured, followed by a 3-week stage of nutritional ketosis induction (Groups 3 and 4) through an intermittent fasting regime: 20 hours of fasting with access only to water, followed by a 4-hour feeding window daily, during which the diet was high in protein and very low in carbohydrates (4:1 protein to fat + carbohydrates ratio). After the study was completed, the animals were sacrificed and blood samples were collected in heparinized tubes, processed for plasma separation, and used for determining standard biochemical parameters.

The entire experimental study protocol followed the regulations of Directive 86/609/EEC, dated November 24, 1986, regarding the protection of animals used for experimental or scientific purposes.

Sample Preparation

At the end of the experiment, each animal was weighed. For the determination of blood glucose, cholesterol, and serum triglyceride levels, blood samples were collected after fasting, centrifuged at 3000 rpm for 10 minutes, and the plasma was separated and stored at -70°C until the biochemical markers assessments were performed.

Determination of Serum Concentrations of Classic Biochemical Markers Involved in the Etiopathogenesis of Metabolic Diseases

The samples from the animals in each group were analyzed, and the following parameters were measured: blood glucose, cholesterol, and triglyceride concentration. Diagnostic kits from Dialab, Austria, were used for the precise determination of these biochemical markers.

Histological Analysis

Histological analysis of the tissues was performed to observe notable tissue and cellular differences induced by nutritional ketosis and the additive effect of melatonin on various organs. Important tissues for the study, such as the brain, liver, kidneys, heart, and adipose tissue, were collected and processed according to protocol. Hematoxylin-eosin-stained smears were prepared and later analyzed under an optical microscope, with interpretations made by a specialist pathologist. Nutritional ketosis was monitored every 2 days after the initiation of intermittent fasting and confirmed through random checks. Urine ketone body detection tests were used to verify ketosis.

Results and Discussion

Table 1. Average values for body weight, blood glucose, cholesterol, and serum triglycerides for each animal group studied.

Investigated Parameter	Group 1: Control - non-obese	Group 2: Obese	Group 2: Obese - Ketosis Untreated	Group 2: Obese - Ketosis Treated
Body Weight (g)	34.25±4.65	42.81±5.34	33.44±3.19	30.89±4.23
Blood Glucose (mg/dL)	80.54±9.15	136.4±8.49	82.14±7.27	79.71±6.63
Serum Cholesterol (mg/dL)	92.94±8.66	132.41±16.56	172.8±16.35	163.37±14.35
Serum Triglycerides (mg/dL)	126.62±11.07	233.3±43.13	231.8±26.16	142.42±15.99

The tabulated values are presented as the means of the 10 values obtained by evaluating each individual within the respective study group.

As can be observed, the body weight of the animals was a conclusive indicator in determining the degree of obesity induction. Following intermittent fasting, body mass showed a significant decrease. Additionally, melatonin administration further accentuated weight loss, with the animals becoming more active and displaying more aggressive behavior in their usual activities.

The carbohydrate metabolism profile normalized following intermittent fasting, as the state of ketosis improved the metabolic characteristics of animals that were previously imbalanced due to the hypercaloric diet. In this case as well, melatonin shows a promising role in the overall regulation of glucose homeostasis, successfully returning the biochemical marker of carbohydrate metabolism to its physiological value.

The lipid profile exhibited a unique characteristic, considering that the animals were on a carbohydrate-restricted diet, which enhanced the mobilization of lipids from adipose stores for conversion into energy. Thus, animals subjected to intermittent fasting showed very high serum triglyceride and cholesterol levels. In the case of co-administration of melatonin, these levels appeared to have a slightly downward trend.

Histological analysis is presented only for the relevant groups and organs, where considerable changes in the appearance and cellular organization of the tissue of interest were recorded.

Histological results for Group 2, with induced obesity, are as follows:

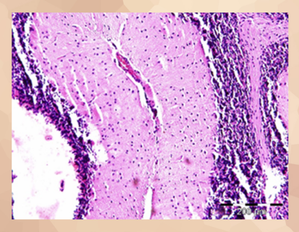

Fig. 1.1. Cerebellum: Edema in the molecular layer with disorganization of Purkinje cells. Vascular hyperemia in the white matter (100x magnification).

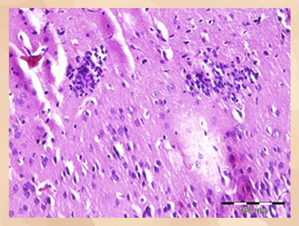

Fig. 1.2. Brain: Mild glial reaction with a nodular appearance in the neuropil (200x magnification).

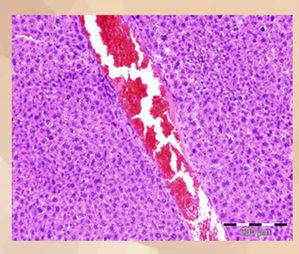

Fig. 1.3. Liver: Edema and hyperemia in the centrilobular vein with a very mild perivascular lymphocytic reaction (200x magnification).

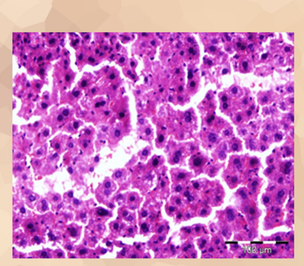

Fig. 1.4. Liver: Edema of the Disse space. Zonal granulovacuolar hepatic degeneration. Hepatocyte nuclei in various stages of karyopyknosis (200x magnification).

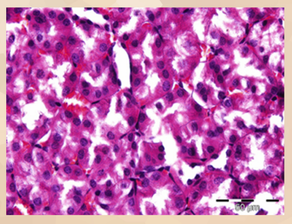

Fig. 1.5. Kidney: Microcenters with disorganization of the intertubular membrane limits. Zonal granulovacuolar degeneration in renal epithelium (400x magnification).

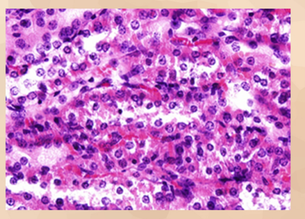

Fig. 1.6. Kidney: Granulovacuolar degeneration in the renal epithelia of the distal convoluted tubules. Zonal pyknosis and renal epithelial cytolysis (400x magnification).

All the highly specific aspects observed in the histological analysis indicate significant cellular damage in individuals with induced obesity, especially in the brain, liver, and kidneys.

Histological analysis for Group 4, which received a diet enriched with margarine, casein, and bread for 3 weeks to induce nutritional obesity, followed by 3 weeks of nutritional ketosis induced through intermittent fasting, and were treated with melatonin, revealed the following relevant results:

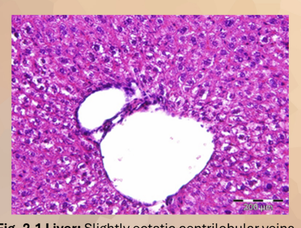

Fig. 2.1 Liver: Slightly ectatic centrilobular veins delineated by Remak cords of hepatocytes, with hepatocytes in different phases of vacuolar degeneration (200x magnification).

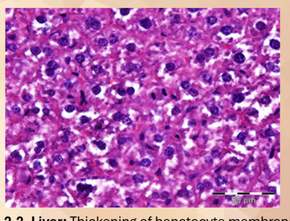

Fig. 2.2. Liver: Thickening of hepatocyte membranes. Uni- and binucleate hepatocytes - present mitoses, absent atypical cells (400x magnification).

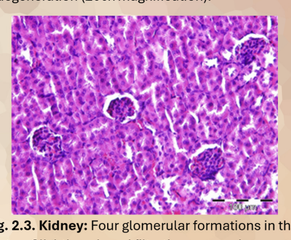

Fig. 2.3. Kidney: Four glomerular formations in the cortex. Slightly reduced filtration space - hyperemia in the capillaries of the glomerular mesangium (200x magnification).

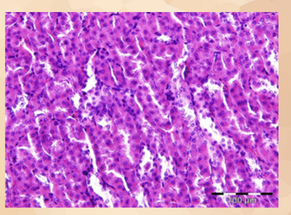

Fig. 2.4. Kidney: Normal appearance of renal epithelia - reduced number of convoluted tubules with disorganization of renal epithelia (200x magnification).

Conclusions

In the case of inducing nutritional ketosis through 20/4 intermittent fasting and administering melatonin, a slight normalization of the functions and morphological characteristics of renal and hepatic cells was observed.

The animals that received melatonin exhibited the following changes: they displayed more aggressive behavior, had discolored feces, the presence of ketone bodies in their urine was confirmed, and the amount of urine decreased significantly.

Thus, using male mice fed a high-fat and carbohydrate-rich diet as a model for obesity, the effect of inducing nutritional ketosis through 20/4 intermittent fasting and melatonin supplementation on preventing obesity-associated complications was investigated through an analysis of the biochemical profile, body mass, and specific histopathological analysis.

Melatonin prevented the increase in body weight and balanced the rise in total cholesterol and triglycerides induced by intermittent fasting, which mobilizes lipids from visceral stores and leads to their metabolism and conversion into energy. These data allow us to deduce that melatonin has a metabolic balancing effect, as it acts to prevent the progression of common biochemical markers, along with reducing obesity.

Nutritional ketosis is an extremely promising therapeutic approach, metabolically rebalancing, which shows a multitude of significant long-term advantages for a range of metabolic disorders.

Song, B.-J. Melatonin Prevents Alcohol- and Metabolic Dysfunction-Associated Steatotic Liver Disease by Mitigating Gut Dysbiosis, Intestinal Barrier Dysfunction, and Endotoxemia. Antioxidants 2024, 13(1), 43
Zhu, Y.; Gao, H.; Lu, M.X.; Hao, C.Y.; Pu, Z.Q.; Guo, M.J.; Hou, D.R.; Chen, L.Y.; Huang, X. Melatonin, an Ubiquitous Metabolic Regulator: Functions, Mechanisms and Effects on Circadian Disruption and Degenerative Diseases. Reviews in Endocrine and Metabolic Disorders 2024
Carocci, A.; Catalano, A.; Sinicropi, M.S. Effects of Melatonin on Cardiovascular Risk Factors and Metabolic Syndrome: A Comprehensive Review. Naunyn-Schmiedeberg's Archives of Pharmacology 2024
Johnson, M.L.; Smith, M.E. Intermittent Fasting Interventions to Leverage Metabolic and Circadian Health Benefits. Am. J. Clin. Nutr. 2023
Hardeland, R. Intermittent Fasting and Obesity-Related Health Outcomes. JAMA Netw. 2023
Tan, D.X.; Manchester, L.C.; Reiter, R.J. Melatonin's Role in Enhancing Ketosis and Fat Metabolism during Intermittent Fasting. MDPI 2024